60 Years of CANCER RESEARCH

From the earliest days of Livermore's history, the Laboratory has leveraged its unique resources and considerable talent against one of the biggest challenges in medicine.

In the early 1800s, the Livermore Valley was rich in ranchland and livestock, finding profit in cattle sales to early gold miners. Over a century later, cattle in Livermore found a very different purpose—helping scientists better understand radiation effects on humans, and in particular workers in the Department of Energy (DOE), from exposure to ionizing radiation and radioactive materials. While humans will always battle new infections and diseases, including the current coronavirus, one constant in our vast range of human health challenges is the fight to cure cancer. The Laboratory's expertise is not explicitly for the benefit of cancer research, but for the past 60 years, the technologies and capabilities built at the Laboratory have advanced our understanding of cancer and carcinogens, from radiation isotope effects in humans to cancer metastasis. As Lawrence Livermore prepares to enter a new decade of cancer research, it builds on a legacy of discovery and innovation marked by its hallmark strengths—specialized facilities, high-precision measurement capabilities, team science, cutting-edge engineering, and computational excellence.

The Nuclear Unknown

The Laboratory's relationship with cancer research began as soon as it was founded in 1952. Following World War II, Lawrence Livermore was created as a second nuclear laboratory to complement existing efforts by Los Alamos National Laboratory. With oversight from the Atomic Energy Commission (AEC), the Laboratory's mission was relatively straightforward: to advance nuclear weapons science and technology. Nuclear weapons research at the time presented a monumental challenge for scientists trying to understand its effects, particularly on human health. Physical reactions like radiation poisoning manifested within days, however, longer-term effects, such as increased rates of neurodegeneration and cancer, would not appear for years. While it was clear that radiation exposure had long-term repercussions in humans, it was not well understood how or why.

To help answer these questions, Lawrence Livermore established the Biology and Environmental Research (BER) Program in 1963 to study the dose per person of radioactive isotopes in the environment as a result of nuclear weapons fallout. Scientists at the Laboratory were tasked with understanding how to judge radiation exposure in humans, as well as how to detect radiation damage. "We had a farm onsite to look at radiation effects in milk, livestock, and plants. We also looked at how radiation damages cells and people…we were trying to get a deeper understanding of radiation's effects on the human genome and DNA," says Acting Biosecurity Center Director Ken Turteltaub.

Scientists suspected that genetic material was particularly susceptible to radiation damage, and they began to look at site-specific DNA mutations in various cells. True to Laboratory form, Lawrence Livermore scientists developed early technologies to help investigate DNA mutations and better understand their connection to diseases like cancer. "The Laboratory has a long tradition of taking on important problems that require a long time to solve. We didn't always have the tools we needed, so a lot of our tools were developed to address our scientific queries," says Turteltaub.

New assays were used to look at genetic changes, and technologies like the cytophotometric data conversion system

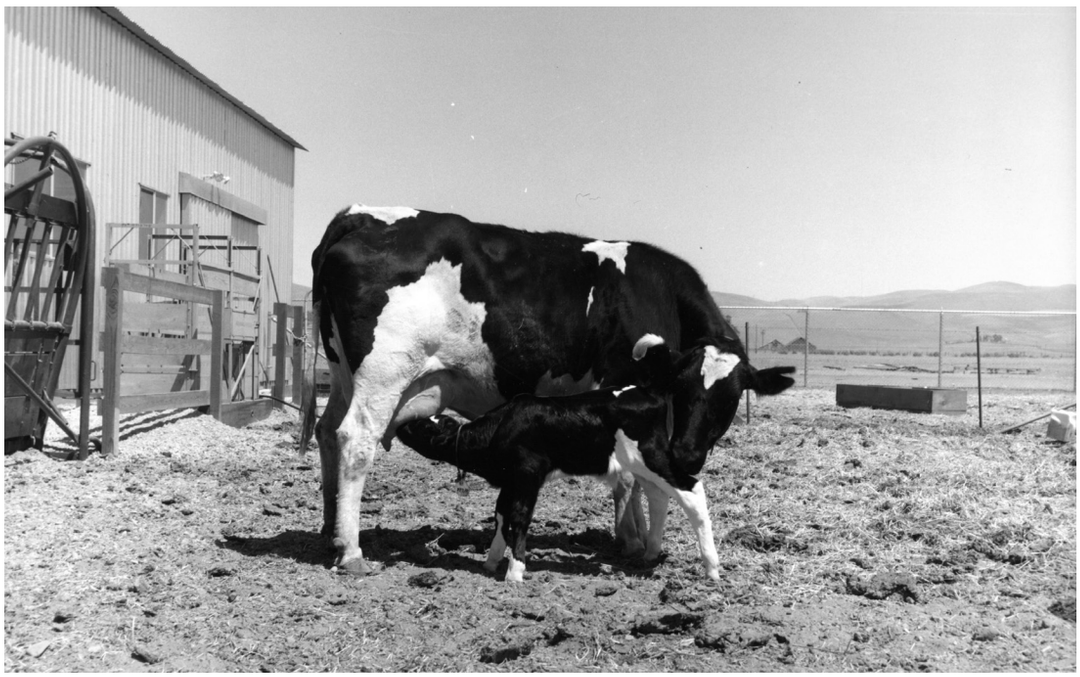
(CYDAC) helped scientists quantitatively examine DNA in any given chromosome. In fact, CYDAC helped confirm a chromosome abnormality in patients with leukemia—these patients consistently experienced a loss of genetic material in chromosome 22 and an excess of material in chromosome 9—an indicator that DNA translocations were connected to cancer.

While cancer research in the 1960s and 1970s was a relatively small part of the Laboratory's overall mission, it was certainly the most talked-about at the dinner table. "Cancer research was actually a very minor piece of total Lab research, but people liked it because it was something you could talk about at home," says Turteltaub.

In 1974, however, facing natural gas shortages, increasing fuel prices, and the lack of a national energy policy, the United States and the national laboratories focused on areas of energy research like fossil fuels and nuclear power.

Despite the Laboratory's pivot from radiation biology, scientists were able to deepen Lawrence Livermore's expertise in DNA and genomics. The cytogenetics lab, led by scientist Tony Carrano, was beginning to use techniques like flow cytometry to measure and sort human chromosomes for the first time. Nearby, Laboratory scientists Joe Gray and Dan Pinkel were developing perhaps one of the biggest biomedical patents to date: fluorescent in-situ hybridization (FISH), used to label and identify genes or chromosomes of interest in a cell. These early accomplishments laid the groundwork for later major research efforts, like the Human Genome Project, which advanced our understanding of cancer even further.

The Laboratory maintained an onsite farm for radiation research in the 1960s, complete with cattle, sheep, peccaries, and two rare pygmy goats. The Holstein cattle pictured composed part of the "Bovinatron" cow barn.

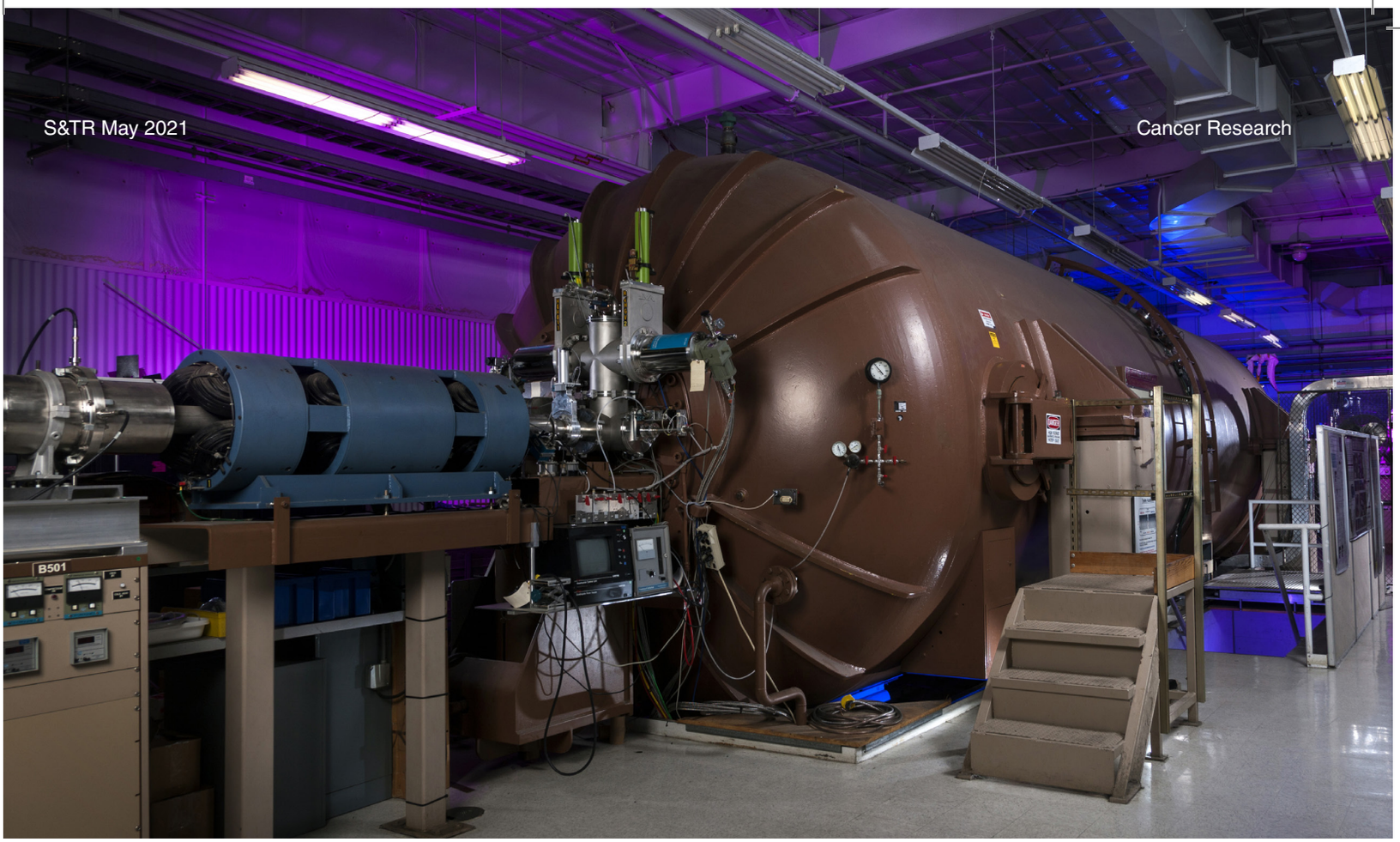


Focus on Food Mutagens

In the 1980s, scientists began to focus on compounds in food that might cause cancer—specifically heterocyclic amines, which form in meat when it's cooked at high temperatures for prolonged periods of time. Bruce Ames from the University of California at Berkeley developed the now famous Ames test—a quick screen for genetic mutation-causing chemicals using a strain of salmonella bacteria—which soon became the standard for determining whether or not a chemical substance was mutagenic. Lawrence Livermore also became involved in this research, focusing on DNA adduct formations, instances where a cancer-causing chemical attaches to a DNA strand and can be used as potential indicators of a human body's exposure to carcinogens. Research in this area was enhanced by the Laboratory's construction and start up of the Center for Accelerator Mass Spectrometry (CAMS) in late 1987—a center designed specifically to study and measure isotope ratios with high precision and sensitivity (see *S&TR*, April/May 2018, pp. 5–11). Turteltaub and John Vogel saw vast potential for the application of CAMS to biosciences, conducting the first biomedical experiments in 1990.

Using ^{32}P-postlabeling and accelerator mass spectrometry (AMS), Turteltaub and his team at Livermore studied DNA damage caused by heterocyclic amine carcinogens found in certain foods. They found early correlations between dose amount and DNA adduction in rodents—and later studies on human subjects confirmed bioavailability of carcinogens with normal levels of dietary exposure.

Lawrence Livermore's use of AMS for biomedical science was a first at the Laboratory and, with support from the National Institutes of Health (NIH), led to CAMS's eventual designation as the official National Research Resource for biomedical applications of AMS. The Laboratory's work on food mutagens contributed significantly to public



The Center for Accelerator Mass Spectrometry (CAMS) has played an important role in Livermore's cancer research efforts since it began operations in late 1987. In biomedical research, CAMS uses an ion beam to determine the ratio of radioactive atoms to normal atoms in organic material. Before CAMS, the Laboratory had never used accelerator mass spectrometry for biomedical science, and its pioneering impact led CAMS to being named the official National Research Resource for such work.

health—informing later-developed food standards and more detailed guidance on how to safely cook meats at prescribed temperatures and durations.

The Human Genome Project

In 1983, Carrano's cytogenics laboratory began its first human DNA library project. The project was led by scientist Marv Van Dilla in partnership with researchers from Los Alamos and represented an early effort to expand the application of flow cytometry to developing fragmented genome libraries based on flow-sorted human chromosomes. The group focused much of their efforts on chromosome 19—a chromosome suspected to have a high gene density—and which also exhibited genes involved in DNA repair. Around the same time, the historic Alta Summit was taking place in the snowy mountains of Utah, where experts nationwide discussed the prospect of using DNA analytical tools to study mutation rates among survivors of the bombings of Hiroshima and Nagasaki nearly 40 years prior. This historical meeting laid the groundwork for the initiation of the Human Genome Project by DOE in 1987, which grew into a partnership with the NIH in 1990 with help from 18 genome centers worldwide. The meeting also led to establishing the complementary Human Genome Center at Lawrence Livermore—directed by Carrano.

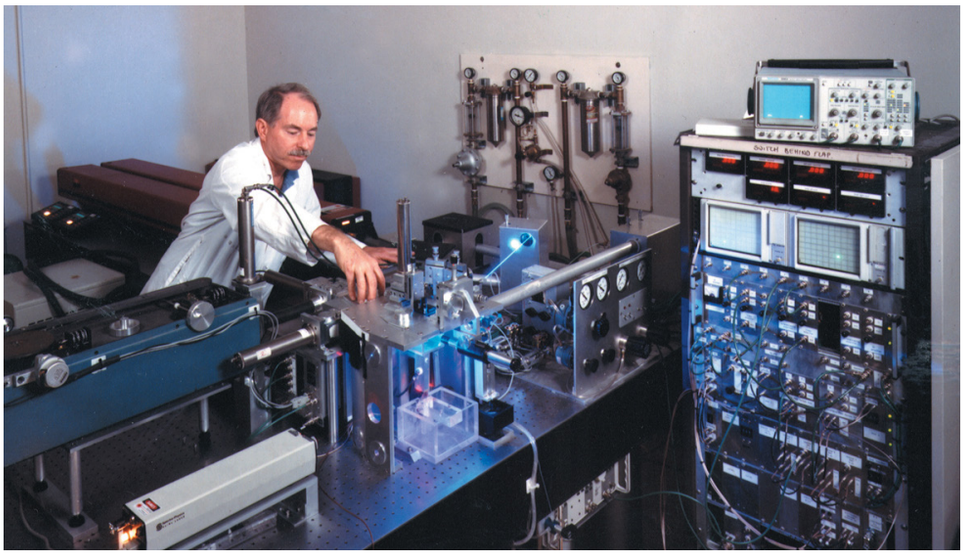
"People thought it would take 100 years to assemble the entire human genome, but DOE wanted to sequence it and LLNL was bringing tools to bear," says Turteltaub. The Laboratory already had the technology to cut and manipulate DNA in addition to other expertise it was able to refine and share with the scientific community at large. Many of the tools cultivated in Carrano's laboratory by scientists like Van Dilla, Gray, and Larry Thompson advanced DNA-relevant technologies including flow cytometry, FISH, and mutant cell development. Flow cytometry, particularly the later-patented MoFlo (modular flow) cytometer, was used to sort chromosomes for the Human Genome Project. The FISH technique, which stained specific parts of chromosomes, allowed for fast detection of aberrations in cells, such as deletions, translocations, or duplications, which are commonly found in cancer cells. Mutant chinese hamster ovary cells cultivated by Thompson provided a cell line that was perfect for building a human chromosome-19 gene library.

While the Laboratory's particular piece of the Human Genome Project was focused on sequencing chromosome 19, perhaps the most significant results of the Laboratory's involvement in the Human Genome Project were the technology and

whole genome libraries it developed in partnership with other laboratories. In the late nineties, Livermore's Human Genome Center joined forces with Berkeley and Los Alamos laboratories to form the Joint Genome Institute (JGI). "JGI helped develop a deeper understanding of the human genome, DNA repair, and how we fix damage," says Turteltaub. "When chemicals or radiation damages your DNA, your body tries to fix it, but sometimes it does this incorrectly and can cause or prevent cancer. Through JGI, we gained a deeper understanding of sequencing and DNA repair which helped us understand how cancer is caused and how to treat it."

The Laboratory's research efforts during this time led to rapid and significant improvements in the supporting technology, particularly in computing devoted to this effort. "Early on, we didn't have a lot of computing and early sequencing wasn't high-throughput. It was experimental. You would compare very specific sequences side-by-side. With the Human Genome Project, it became clear we needed better computing capabilities," says Turteltaub. Computers ultimately helped fill the gaps that human minds could not. "Sequencing is not linear, so computers helped align DNA and see the overlaps. Over time, the pieces became longer and we developed more research queries, like identifying protein structures. Research evolved from DNA alignments and comparing sequences to physics informatics and looking at mutations in proteins," explains Turteltaub.

The first draft of the human genome was published in 2001, and the project was formally completed and published in 2003. In this time period, the Human Genome Center at Livermore developed and maintained an extensive database for chromosome 19 information, as well as a library, which enabled the cloning of specific genes for delivery to other scientists. Laboratory scientist Lisa Stubbs enhanced this effort through a successful program to annotate and assign biological functions to genes harbored on chromosome 19. She focused on a particular cluster of transcription factors, determining how they evolved and diverged across species.



Early groundbreaking work in flow cytometry, a technique for separating specific cells from other cells, has led to numerous medical research applications in genomics research and national security applications, such as biosensors that detect specific agents used in biological weapons.

As part of the JGI, Livermore contributed to the full sequencing of chromosomes 5, 11, 16, and 19. While the JGI still operates today, Livermore is much less involved. Its contributions to sequencing, genome assembly, gene annotation, and other technologies, however, left it well-positioned for cancer research efforts nearly two decades later.

Cancer Moonshot

Due to funding shifts and sponsor directives, the Laboratory's cancer research was scaled back in the early 2000s, and the biology focus shifted to biosecurity. In 2016, however, that changed with the initiation of the Cancer Moonshot program. A year prior, President Barack Obama issued an executive order to establish the National Strategic Computing Initiative (NSCI)—a multiagency effort to find new ways to apply high-performance computing to scientific discovery. One of these approaches emerged through conversations between DOE and the National Cancer Institute (NCI). NCI expressed a need to advance oncology with computational and data analytics with three levels of research: cellular, molecular, and demographic. While serving as vice president in 2016, Joe Biden announced the National Cancer Moonshot—a concerted effort to discover a cure for cancer, which took the life of his son Beau in 2015.

The Moonshot involves multiple initiatives, including the Joint Design of Advanced Computing Solutions for Cancer (JDACS4C), which is a pilot pursued by NCI and DOE (see *S&TR*, November 2016, pp. 4–11). As part of JDACS4C, Lawrence Livermore works with NCI and its Frederick National Laboratory for Cancer Research (FNLCR), and with Los Alamos, Oak Ridge, and Argonne national laboratories to tackle three pilot programs.

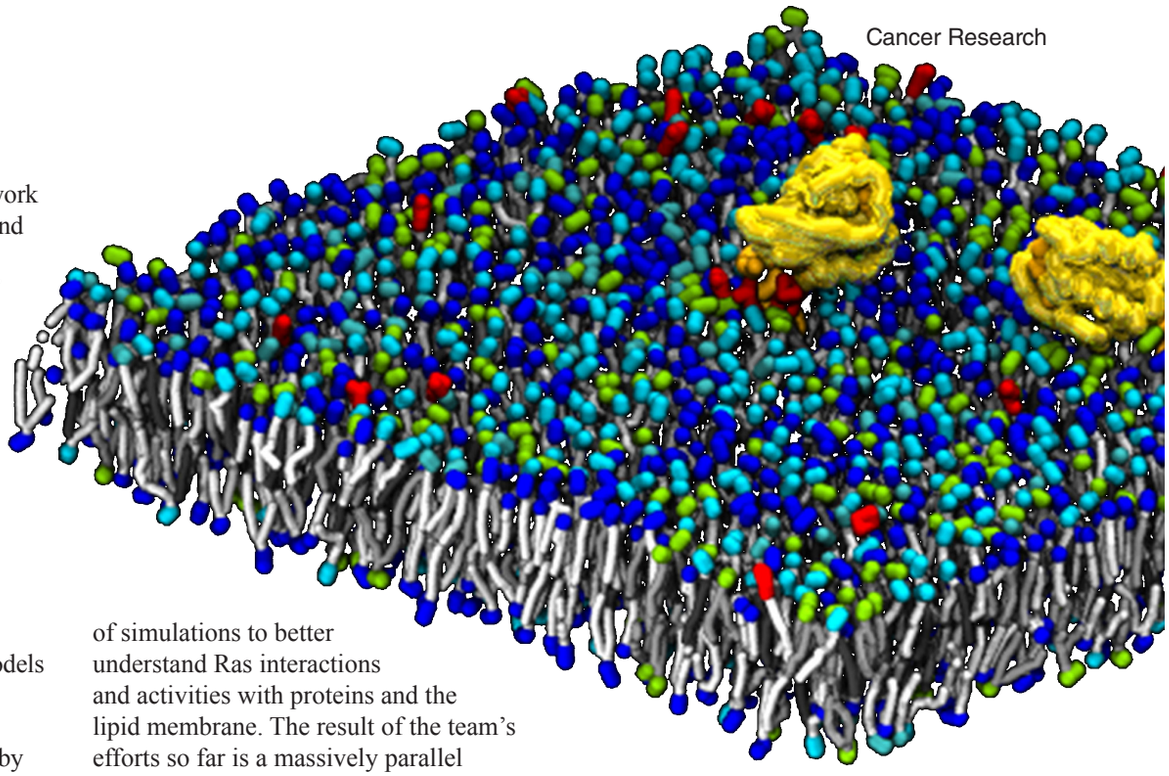
Amy Gryshuk, who at the time served as the program development liaison and now leads the Laboratory's Physical and Life Sciences Directorate Strategic Science Engagements Office, and Eric Stahlberg,

representative for FNLCR, coordinate work between the DOE labs and NCI. "Eric and I facilitate collaborative conversations and identify new growth opportunities," explains Gryshuk. "With so many moving parts, it's important to understand the dynamics of each partner and how we can leverage each national laboratory's strengths for the pilots' successes." This close-knit partnership has been critical to the pilot programs' accomplishments so far.

The first pilot, led by Argonne, NCI, and FNLCR, focuses on identifying new treatments using computations and developing and validating predictive models which can "grow" a patient's tumor and guide therapeutics based on modeling predictions. The second Ras pilot is led by Fred Streitz of Lawrence Livermore and Dwight Nissley of FNLCR. It endeavors to gain a deeper understanding of cancer biology, starting with the Ras protein which plays a critical role in cell growth and is often at the source of cancer development. Finally, the third pilot, led by Oak Ridge and NCI, is focused on cancer surveillance and applying computation to find patterns in cancer-based data, including diagnostics, treatments, and individual patient factors. Los Alamos's role touches all three pilots—providing uncertainty quantification for machine learning to better understand predictive models and simulation reliability.

For the Ras pilot, the Laboratory has made significant contributions in computing and predictive models to better understand cancer development. The research involves a close synergy between experiments and computation, allowing scientists to get a better picture of the Ras protein and how it interacts with a cell's lipid membrane. "Ras is the undruggable protein. We want to target it specifically because oncogenic mutations of Ras are implicated in some of the most aggressive cancers we know, such as pancreatic and colon," says Gryshuk.

Using high-performance computers, like Lawrence Livermore's Sierra machine, scientists have run tens of thousands of simulations to better understand Ras interactions and activities with proteins and the lipid membrane. The result of the team's efforts so far is a massively parallel Multiscale Machine-Learned Modeling Infrastructure (MuMMI) that has helped scientists find relevant seed data for further modeling and analysis of Ras protein interactions.



Lawrence Livermore researchers led a multi-institutional collaboration to develop a machine learning-based simulation capable of modeling the protein interactions and mutations that play a role in many forms of cancer. This micro-model simulation shows the aggregation of Ras proteins (yellow) on top of a cell membrane model. (Rendering by Timothy S. Carpenter.)

As JDACS4C enters its fifth year, Gryshuk reflects on the importance of interagency collaborations—one of the pillars of team science. "I don't think there's been an interagency collaboration at this level before. This collaboration has allowed us to share resources, integrate experimental and computational efforts in an iterative form, and gain valuable access to clinical data, which national laboratories don't normally have access to. We're using computing to identify potential treatments, better understand cancer biology, and analyze cancer-based data. We're continuing to grow in partnership," says Gryshuk.

Past Meets Present

The Laboratory's current cancer research efforts span a range of departments, disciplines, and focus areas—a unique fact considering cancer research is not, and never was, one of the Laboratory's core mission areas. "Cancer research isn't spelled out in our core mission. It can be broadly categorized under health security," explains Biosciences and Biotechnology division leader Kris Kulp. "The role we play is that we take advantage of the strengths of the Laboratory. We offer computing and new experimental techniques to help investigate cancer research questions," says Kulp.

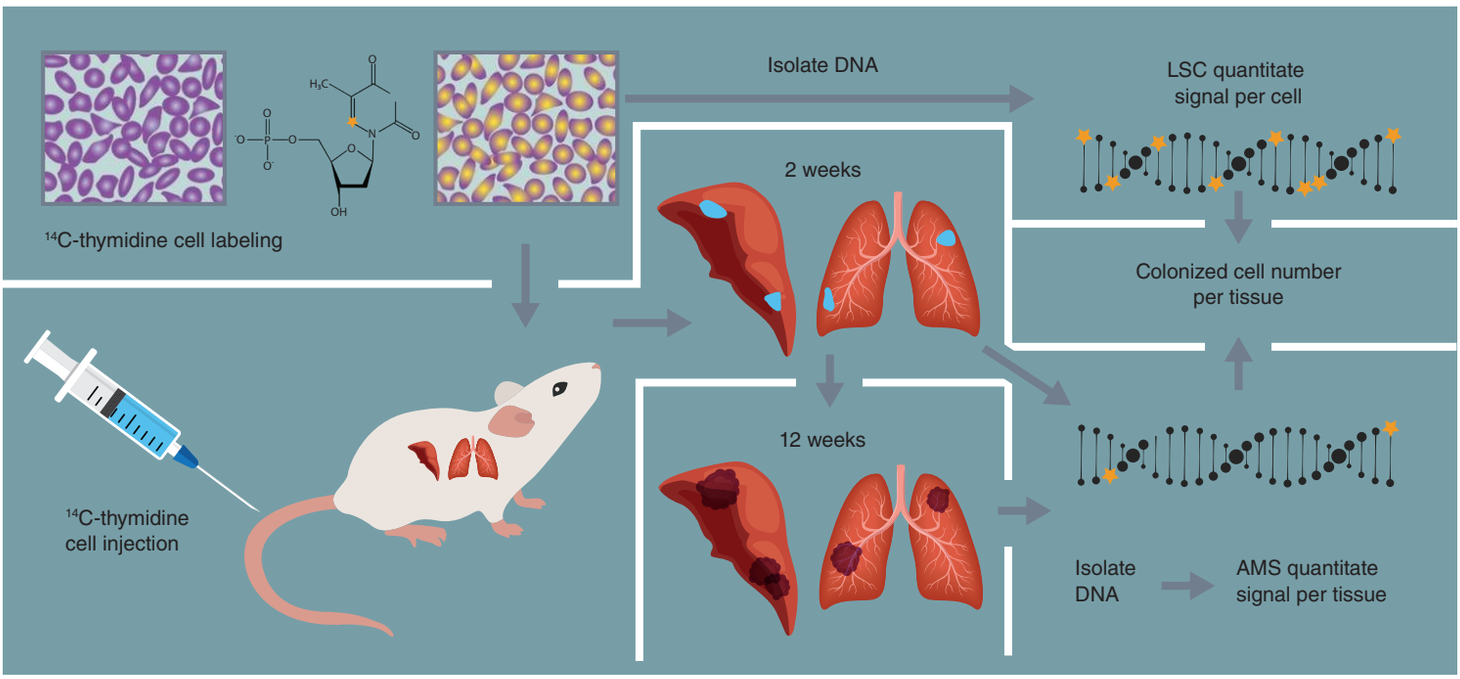
The laboratory of Lawrence Livermore scientist Gaby Loots is one place where experimentation, technology, and diverse expertise intersect on a regular basis in support of cancer research. Loots came to the Laboratory 18 years ago with a degree in genetics and genomics, seeking to better understand the function of DNA regions that are evolutionarily conserved and control transcription of genes. She now leads a successful program in bone and cartilage research, examining the risk factors that contribute to degenerative disorders like osteoporosis and osteoarthritis. Cancer

research became a natural secondary research area in her lab because cancer metastasis—when cancer spreads to other parts of the body from its origin site—spreads to bone in most aggressive forms of the disease. "We started cancer research 10 years ago, and we noticed a lot of the signaling pathways active in bone are also up-regulated in some cancers, so we studied the process of cancer metastasis to bone. We wondered, 'What is it about the bone environment that allows cancer to thrive?'" says Loots. Once cancer has metastasized to bone, it becomes much more challenging to treat and rapidly increases mortality. "Cancer adapts quickly and can block the immune system. When the balance shifts in favor of cancer, there's little chance of going back."

One way Loots and her laboratory team study cancer metastasis is by developing innovative ways to track cells and small secreted vesicles, called "exosomes." In an effort led by scientist Nicholas Hum, the team recently found a new way to quantify the number of cells that initiate metastatic tumors using the Laboratory's BioAMS signature facility. "We label cancer cells ex vivo with ^{14}C-thymidine, and the chemical incorporates into the cell's DNA and creates a signature that can be tracked in the body. With this approach, we can estimate the number of cancer cells that migrate to a distant organ and quantify how many cancer cells initiate a metastatic tumor," explains Loots. The AMS is able to accurately measure the initial number of ^{14}C-labeled cells that formed a tumor in a tissue after defined periods of time because of the long half-life of ^{14}C. The method also helps scientists determine if tumor cells have been completely removed in response to treatment. "Remission is a huge issue—after treatment, sometimes cancer cells remain dormant and undetectable for months or years, but when they reignite, they form drug-resistant tumors that readily metastasize. Our method allows us, with very high precision, to see if any cancer persists or has been eradicated," says Loots.

The team relies heavily on AMS to both track cancer cells and label chemotherapeutics to observe their success in treating cancer. One way their team is approaching chemotherapeutics research is via microdosing—an effort started by Turteltaub. "Microdosing allows you to give a small amount of therapeutics and determine the amount of drug present in cells or bound to DNA, which usually correlates with chemo resistance or sensitivity. We use AMS to determine how many molecules of the drug actually make it to a tumor," says Loots. Microdosing is important because it

By injecting mice with a 14C-thymidine radioisotope, CAMS researchers examined radiation's effect on cell development. Tissue was harvested at 2 and 12 weeks to show different stages of growth, and the mouse DNA was isolated and quantified using AMS. In parallel, cultured DNA was quantified with liquid scintillation counting (LSC), a method that mixes organic material with a fluorescing solvent and measures the resultant photon output. The AMS and LSC results were then combined for a calculation of how many cells in each organ had been colonized with cancer cells.



Scientists in Livermore's gene library project prepare to ship out one of the first gene libraries in 1984. From left, (first row, seated) is Lil Mitchell, Nancy Allen, and a summer intern. Second row: Lynn Minker, Tony Silva, Linda Ashworth, Janine Perlman, Lo Chung Yu, and Jim Fuscoe. Third row: Anthony Carrano, Karla Henning, Marvin Van Dilla, Leilani Corell, Mortimer Mendelsohn, Joe Gray, Dan Pinkel, and Don Peters.

facilitates a Goldilocks approach to chemotherapy treatment—too much or too little can actually hurt a patient. "In a petri dish, any drug can seem effective, but you have to balance the toxicity of the treatment with its efficacy. It may look like it's killing 100% of the cancer but it may also be killing healthy cells, making the patient sick," says Loots.

Determining pharmacokinetics is a key application of AMS. "You can label any drug with ^{14}C and determine its uptake, distribution, and clearance, including how long drugs stay in the system. We can also look at factors such as whether or not the drug is crossing the blood–brain barrier. AMS helps you quantify trace amounts of a drug in different tissues," says Loots. The Laboratory's extensive use of the AMS is part of what makes its cancer research unique. Kulp explains, "We have a long history of using the AMS for cancer questions. Our team includes world experts in AMS, and the facility is a really compelling reason for us to do cancer research." Another key element of the AMS is collaboration—it's currently set up as a user facility with support from the NIH, so other laboratories and organizations can also take advantage of its unique capabilities.

Also critical to the Laboratory's cancer research is its partnership with local universities. The Loots lab specifically leverages its partnership with the UC Davis Comprehensive Cancer Center (UCDCCC) to bring in graduate students and postdocs, as well as share resources. "We are a consortium with UCDCCC—I'm essentially a matchmaker between UC Davis and Livermore. They can use the Laboratory's technology, and we can have access to human samples and reagents, which we wouldn't have access to otherwise," says Loots.

Research for the Cure

Other exciting, current cancer research areas include scientist Monica Moya's research into cancer metastasis using three-dimensional bio-printing. Her project involves engineering human tissues to mimic actual biological functions, which would potentially allow scientists to model how cancer moves through blood vessels without having to test therapeutics on humans or animals. Scientist Claire Robertson is using a similar method to look at cell communication during the progression of breast cancer. Another effort, led by scientist Sean Gilmore and funded by the Laboratory Directed Research and Development program, is focused on nanometer-scale, particle-based immunotherapy for cancer treatment. Gilmore's project is investigating use of a combination of chemotherapy and immunotherapy to treat cancer that takes advantage of the natural pharmacokinetic properties of nanometer-scale particles, like nanolipoproteins.

From researching the effect of radioactive isotopes in humans, to food mutagens, to understanding the human genome and cancer metastasis, the Laboratory's relationship with cancer research has continued to evolve with new technology and information. Livermore's efforts benefit both the Laboratory's national security mission and the mission closer to home: human health. Says Loots, "Any animal models we generate are directly applicable to national security, to developing countermeasures to malicious chemical and biological agents, and to improving resilience in our military personnel. We can use the same approaches to determine harmful effects of pathogens as we do to study mechanisms of cancer development. Our methodologies and infrastructure always have the mission at heart." As the Laboratory enters a new decade of cancer research, it will continue to apply its range of unique resources and expertise until one day cancer is cured.

—Lauren Casonhua

Key Words: accelerator mass spectrometry (AMS), bio-printing, cancer metastasis, cancer moonshot, cancer research, chromosomes, cytophotometric data conversion system (CYDAC), DNA adducts, flow cytometry, fluorescent in-situ hybridization (FISH), food mutagens, gene library, genomics, heterocyclic amines, high-performance computing, Human Genome Project, Joint Design of Advanced Computing Solutions for Cancer (JDACS4C), Joint Genome Institute (JGI), MoFlo, Multiscale Machine-Learned Modeling Infrastructure (MuMMI), National Cancer Institute (NCI), National Institutes of Health (NIH), predictive models, radioactive isotopes, Ras protein, Sierra supercomputer.

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